GAMETOGENESIS IN THE OYSTER UNDER CONDITIONS OF DEPRESSED SALINITY

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The American oyster, *Ostrea virginica* Gmelin, flourishes naturally in brackish waters ranging in salinity from 16 to 27 parts per thousand. But the salt toleration of the animal is such that it can survive in waters having a much broader range of salt content. In many localities, commercial production of oysters is maintained where seasonal floods may expose the bars to entirely fresh water for short periods of time. Some of the more important seed-producing areas on the Atlantic Coast consistently have a salt content of less than 15 $^0/_{00}$. Consequently the effects of lowered salinity on oyster physiology and reproductive ability have long been of interest. The opportunity presented itself in 1946 to examine the gonads of oysters living under unusually great variations in salt content. Extensive flood waters from the Susquehanna River watershed into the upper reaches of Chesapeake Bay during the summer of 1945 and spring of 1946 caused salinity depressions from a normal range of 10 to 15 $^0/_{00}$ to zero for protracted periods. Oyster beds located twenty miles south of the entrance of the river into the bay were frequently exposed to fresh water. In the period following these extremes, mortalities up to 70 per cent of the population were recorded on the bars in this area (Engle, 1946). The oysters remaining viable were of unusually poor quality. The body tissues were edematous and nearly transparent. The adductor muscle lacked tonus so that the valves could be separated easily and frequently were gaping.

Samples of ten or more oysters from this low salinity area, designated here as the LS group, were collected weekly in the summer and at longer intervals during the fall and winter of 1946. Transverse sections of the gonad were prepared for histological examination. For comparative purposes, a similar series of oysters, designated as the HS group, was collected in another part of the bay where the salinity was higher and remained relatively unaffected by the flood conditions. These oysters were of good market quality and during the summer produced a set of young oysters of commercial proportions indicating normal gonad development and spawning reactions. Routine hydrographical observations were made at the time of each sampling, as well as plankton tows and notes on the feeding activity and general condition of the oysters.

Of the 185 oysters in the LS group over three years old examined, 40 per cent were females, 33 per cent were undifferentiated, 26 per cent were males and 1 per cent were sex reversals. Of the 221 specimens in the HS group, 70 per cent were females, 29.5 per cent were males and 0.5 per cent were hermaphroditic. The absence of undifferentiated gonads in the HS group was striking in comparison with the LS group.

The orderly sequence of events in the development of functional gametes in the American oyster has been described (Coe, 1932; Loosanoff, 1942), and the résumé of the stages given here for the HS oysters growing in Chesapeake Bay differs in no important respect from conditions found elsewhere except with regard to timing (Loosanoff and Engle, 1940). Spawning is initiated when water temperatures rise to levels approximating 18 to 20° C., and consequently its occurrence varies from year to year at any particular geographical location. Typically, after the final spawning of the population in late summer, there is a short period of rest in which the gonadal tissue is made up of undifferentiated gonial cells. These soon proliferate and early maturation takes place. By this time, usually late December in Chesapeake Bay, water temperatures have decreased to the extent that the oyster becomes inactive and the gonad remains quiescent until the following March. Thus in early spring, gonad sections from the HS oysters are characterized by fairly large numbers of auxocytes. As water temperatures increase, differentiation and growth proceed at a rapid pace, and mature gametes first appear in May when spawning may begin. In June most of the gonads are filled with ripe sexual products, and from that time until early September, successive waves of spawning may continue. By the end of September the majority of gonads are in the resting condition.

In contrast to this typical picture, section of the gonads of the LS group revealed that 5 to 40 per cent of each sample contained gonads which were in the resting

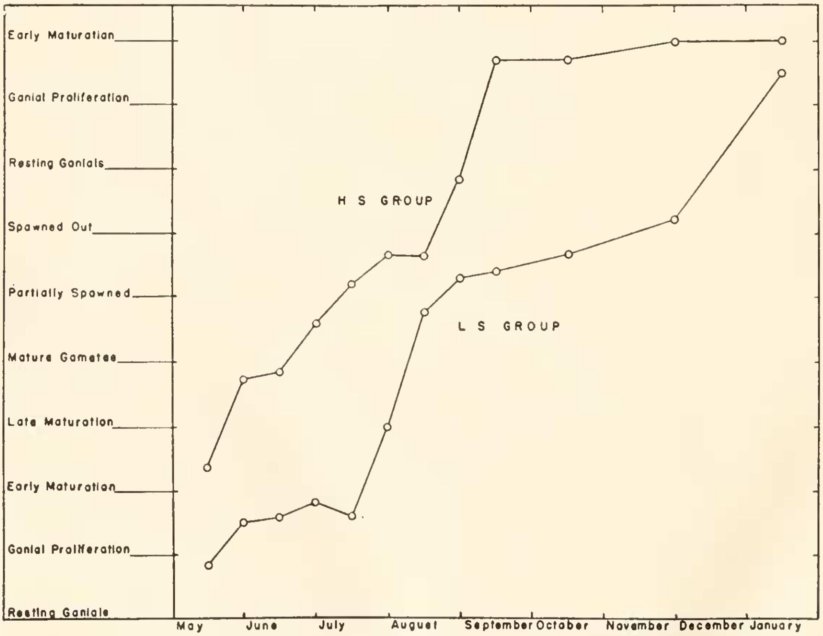

FIGURE 1. Seasonal progression of stages in the development of gametes in oysters from a low salinity area (LS Group) and from a higher salinity area (HS Group). Each point represents the predominant activity in a sample of 10–40 oysters collected during a two-week or longer period. Initial and final phases in the growth of auxocytes, termed Early and Late Maturation, are normally separated by the winter hibernating period in this area.

gonial or undifferentiated stage until the middle of August. This condition must have persisted from the close of the spawning period of the previous year, the time of its normal occurrence. By the end of August there was a marked improvement in the appearance of the oysters, and the gonads reached stages of activity which had characterized the HS oysters examined two months earlier. In early November, a majority of the LS oysters were spawned out, and from this time until January, early maturation continued at a high level. When the oysters finally entered the hibernating stage at temperatures of less than 5° C., the majority of LS oysters were indistinguishable, with respect to the histology of the gonad, from the oysters living in the higher salinity area.

In about 90 per cent of the specimens of LS oysters examined, the gametogenic cycle lagged approximately two months behind that of the high salinity group, but in the remaining 10 per cent of the specimens, the gametogenic cycle showed the same timing pattern as in the HS group. In order to portray graphically the differences between the two populations, the successive stages in normal gonad activity were assigned arithmetic values from one to ten, depending on the preponderant condition or cell type present. The average arithmetic value was then obtained for each sample of gonads collected over a two-week or longer period and has been plotted against time for the two areas studied (Fig. 1).

It was observed that during the summer, developmental stages in different gonads of a sample overlapped or were concurrent because of the relatively long period of four months in which eggs are produced. The average values shown in Figure 1 demonstrate the seasonal trend of the gametogenic cycle, but they do not show the wide variations found within each of the samples of oysters. Earlier investigators (Nelson, 1928; Loosanoff, 1942) have noted the variations in gonad response found in some individuals of a sample where, for unknown reasons, maturation may be delayed or physiologically mature gametes may be retained long after the general population has spawned. This condition is especially prominent in the LS oysters examined. In the first week of August, individuals from one sample demonstrated all stages in gonad development from the undifferentiated gonial cells to the spawned-out stage. The degree of variation among individuals was far less extensive in the HS group, in which for the same period the gonads were fairly equally divided between the partially spawned and the spawned-out stages. The percentage distribution of each stage within the samples collected is tabulated to illustrate this disparity in the two populations (Fig. 2).

During the first two weeks of August there was a significant change in the appearance of the gonad sections from the oysters of the LS group. Wide variations in the stages of activity attained by the individual oysters continued, but all of the gonads suddenly advanced beyond the indifferent and early maturation stages, and 50 per cent of them were partially or almost completely spawned. In this period there were only minor fluctuations in the temperature but the salinity rose abruptly from less than 3 ‰ to more than 8 ‰. No other environmental changes of importance were noted during this period.

The recovery of oyster larvae from the plankton tows made at the two stations corresponds, in general, with the histological picture.[1] In the high salinity area,

[1] The writer is indebted to Mr. James B. Engle of the U. S. Fish and Wildlife Service, who provided the data on plankton.

larvae were found two weeks after mature gametes were observed in the sections. The last plankton sample taken, October 8, still contained numerous larvae, although it was from two to three weeks after the apparent absence of gametes from the gonad sections. There were two seasonal peaks in larval production: the first week of July and the last week of August. In the low salinity area there was but one seasonal peak toward the end of August. No larvae at all were collected here until seven weeks after their initial appearance in the HS area, although throughout this period 10 per cent of the gonad sections had contained apparently mature gametes. The failure to find larvae in the water at that time may be attributed to inadequate sampling methods or, more probably, to the inhibition of spawning. The observed tissue edema may have interfered with the activity of the adductor muscle in the spawning reaction (Galtsoff, 1938) or have partially closed the gill ostia, thus preventing the passage of ova to the exterior (Hopkins, 1936).

	May 16-31		June 1-15		June 16-30		July 1-15		July 16-31		August 1-15		August 16-31		September 1-15		September 16-30		Oct		Nov. & Dec		Jan	
	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H	L	H
EARLY MATURATION																25	9	74	11	75	10	100	55	100
GONIAL PROLIFERATION																5		21	11	20	30		45	
RESTING GONIALS														4	9	10	9	5		5				
SPAWNED OUT								10		20	16	63	30	55	9	50	9		11		20			
PARTIALLY SPAWNED							6	37		80		37	20	41	82	10	55		56		40			
MATURE GAMETE		11	7	75	16	84	12	53	24		47		50				18		11					
LATE MATURATION		39	7	25		16																		
EARLY MATURATION	32	39	36		32		35		21		5													
GONIAL PROLIFERATION	21	11	29		26		41		21		5													
RESTING GONIALS	47		21		26		6		34		27													

FIGURE 2. The percentage distribution of different stages of gonad activity in each sample of oysters collected from the low (L) and high (H) salinity areas. See legend under Figure 1 for description of samples.

Water temperatures throughout the period of observations were normal for the region (Fig. 3). In the low salinity area, the bottom temperature was 16.8° C. in the middle of May, approached 20° C. in the first week of June, and reached the summer maximum of 26° C. on the first day of August. It then decreased gradually to 15.8° C. at the end of October, and to less than 5° C. in the period December through January. Bottom water temperatures in the high salinity area regularly followed the same levels within one or two degrees.

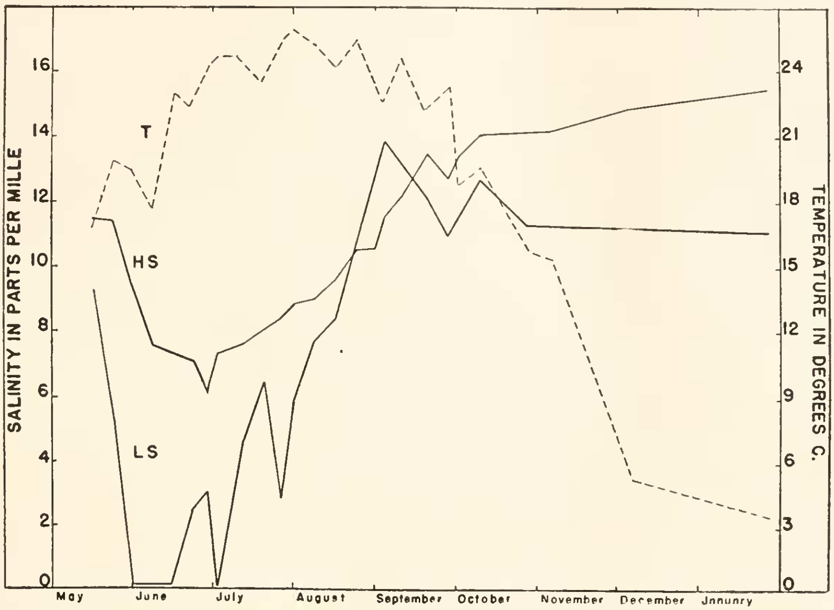

FIGURE 3. Seasonal fluctuations in salt content of bottom waters in the high (HS) and low (LS) salinity areas. The bottom temperature curve (T) is shown for the LS area. Temperatures in the HS area did not vary more than one degree plus or minus from these data.

The salt content of the water was more variable (Fig. 3). In the LS area, bottom salinities fluctuated from zero (fresh) to 6 $^0/_{00}$ in the period from the middle of May until the first of August. One-third of the records for this time showed fresh water. In August the salt level increased steadily to 13 $^0/_{00}$ and then gradually dropped to 11 $^0/_{00}$ by the end of the year. In the HS area, the lowest salinity of 6 $^0/_{00}$ was recorded toward the end of June. Before and after that time, the salinity increased steadily to 15 $^0/_{00}$.

Only four specimens, one per cent of the total examined, gave evidence of the instability of the sex mechanism in this species of oyster. Three of the individuals were clearly defined protandric reversals in which the gonaducts contained residua of spermatozoa, and the walls of the follicles were lined almost exclusively with oocytes in early stages of maturation. These specimens were obtained early in September, which indicates that reversal of sex had taken place when the majority of the population were spawning. Loosanoff (1942) has suggested that sex reversal takes place when the gonads are made up of undifferentiated gonial cells, usually in late October in Long Island Sound. It would appear most reasonable that sex reversal should take place during this stage, but the specimens found here suggest that if it does, the indifferent stage may occur much earlier in the summer, i.e., July and August, in at least part of the population. In one of the four specimens mentioned above, the gonadal tissue was made up of fairly equal numbers of developing oocytes and spermatocytes within each follicle. The developmental stages attained were the same as in other unisexual specimens collected at the same time, indicating that this oyster would have been a functional hermaphrodite when the general population spawned. The percentage of intersexes found in this rather small sampling agrees with observations by other workers. In the 221 oysters from the HS area there was a ratio of females to males of 2.41. This figure is comparable with observations in Galveston Bay, Texas, but contrasts with the approximately 50–50 sex ratio found along the Atlantic Coast (Hopkins, 1931).

The deleterious effect of the environment on the physiology of the oyster, as evidenced by the delayed production of gametes until such time that the water temperatures made their survival improbable, would appear to be due to the low salinity of the water. That this effect was not a direct inhibition of gametogenesis is indicated by the fact that 10 per cent of the LS group elaborated mature gametes at the usual time in the early summer. The factors directly affected by lowered salinities which may be operating here to prevent gametogenesis include several possibilities. It has been shown (Hopkins, 1936) that during exposure to fresh water the oyster's valves may be closed most of the time and also that even when open, the passage of water through the gills may decrease or stop entirely. Either one or both of these factors would seriously curtail the feeding of the animal. It is also possible that during this time necessary food elements were absent from the plankton, or that tissue edema prevented the normal assimilation of food. In any event the end result appears to have been, fundamentally, a tissue starvation. Hopkins (l.c.) theorized such an end result after studying the feeding mechanism in *O. gigas* in the presence of artificially lowered salinities. It was noted that in the small group of LS oysters which produced gametes at the usual time in late spring, there was a moderate reserve of stored food which gave the tissues a typical opaque appearance. These oysters, as well as the ones having no visible food storage, had empty digestive tracts at the time of examination. This would indicate that the reserve food had been held over from the previous fall rather than that this small group had been able to continue feeding during the period of lowered salinities. The evidence is clear, moveover, that soon after the salinity level rose above 6 $^0/_{00}$ in the first week of August, the animals commenced feeding, there was an obvious improvement in the appearance of the tissues, and gonad activity started to approach the normal picture.

Summary

Histological examination of oyster gonads from an area naturally exposed to prolonged periods of fresh water, when compared to oyster gonads from an adjacent, unexposed area, showed:

1. Gametogenesis was inhibited in 90 per cent of the surviving population until salinity levels rose above 6 parts per thousand.

2. Following the salinity increase, oysters rapidly improved in condition but required from three to four months to attain the same final level of gonad activity as the unaffected group.

3. Marked variation and suppression of gonad activity in the exposed oysters is attributed to variations in food availability, rather than to direct inhibition of sexual activity by less saline water.

4. Sex ratios and extent of intersexuality in the population sampled, as well as details of the gametogenic cycle, agree for the most part with published observations on *Ostrea virginica* in other parts of its geographical range.

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